Aquatic Toxicology Laboratory
33972 Texas St. SW
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Office 541-737-2565
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Oregon State University OSU

1195583

Project Title: Pilot Study: Evaluation of free-fiber Libby Amphibole (LA) asbestos toxicity in laboratory water to the rainbow trout (*Oncorhynchus mykiss*)

Testing Facility: Oregon State University Aquatic Toxicology Laboratory (OSU AquaTox)
33972 Texas St. SW
Albany, Oregon, USA 97321

Study Sponsor: Golder Associates, Inc.
18300 NE Union Hill Road, Suite 200
Redmond, WA 98052

Principal Investigator/ Study Director: William Stubblefield, Ph.D., Oregon State University

Revision Number	Date	Reason for Revision
0	30 November 2010	--
1	06 December 2010	Incorporate comments
2	22 April 2011	Incorporate amendments
3	28 April 2011	Incorporate Golder/Remedium Comments

1.0 INTRODUCTION

1.1 Objective

To conduct a pilot study to evaluate the feasibility of conducting a toxicity test with free-fiber Libby Amphibole asbestos (LA) and to determine the maximum duration that LA remains as free-fibers under laboratory toxicity test exposure conditions.

1.2 Experimental Approach

Rainbow trout (*Oncorhynchus mykiss*) will be exposed to varying concentrations of LA. Aliquots of water will be sampled at test initiation (time zero) and every 24 hours, for a total of 5 days, to determine the maximum length of time between the existence of LA as free-fibers and the removal of fibers from the water column due to clumping and binding processes.

1.3 Test Substance

Libby amphibole (LA) asbestos will be supplied as concentrated stock solutions. Ampoules of 15 mL of concentrated stock, prepared for each exposure concentration, will be added to 15 L of dilution water to achieve each final nominal exposure concentrations. The United States Geological Survey (USGS) will provide raw LA material to the analytical laboratory who will then prepare concentrated stock solutions, as directed by the protocol titled, "Protocol for the preparation of ampoules for use in toxicity testing of Libby Amphibole to fish" (Ampoule prep protocol v3.doc; Attachment 1). The ampoules will be shipped from the analytical laboratory to OSU AquaTox.

2.0 BASIS AND TEST SYSTEM

2.1 Basis

This protocol is designed to comply with decisions made by the Libby Asbestos Site Operable Unit 3 Biological Technical Assistance Group (BTAG) on bioassay testing and analytical studies at OU3. This "pilot study" will determine whether fish can be consistently exposed to nominal concentrations of free fibers of LA without significant free fiber loss to clumping/binding, based upon the proposed study design.

2.2 Test Organism

1. Species – Rainbow Trout (*Oncorhynchus mykiss*)
2. Number – Each exposure concentration will consist of 45 organisms (15 fish in each of the 3 replicates)
3. Age – *O. mykiss* will start as juvenile fry (~1-2").
4. Source – *O. mykiss* will be obtained from Trout Lodge (Sumner, WA, USA).
5. Culture/Holding Water – Upon arrival at the OSU AquaTox Laboratory, fish will be slowly acclimated to water temperature and water conditions. *O. mykiss* will be maintained in a continuous flow-through system and be held for up to 2 weeks prior to test initiation.
6. Feeding – Each chamber will be fed a trout chow slurry twice daily at a growth maintenance rate of 4% of their body weight. Mass of fish at test initiation will be

determined using a surrogate batch of fish which were maintained with the fish to be used in the study.

7. Procedure for identification: *O. mykiss* will be verified to species by the supplier.
8. Justification: This testing protocol follows recommendations made from the Libby Asbestos Site Operable Unit 3 Biological Technical Assistance Group (BTAG).

2.3 Test Diet

Starter-grade trout chow (Bio Oregon, Warrenton, OR) is combined with reconstituted moderately hard lab water to produce a slurry for feeding. The slurry is made by combining 1.2 grams trout chow with 10 mL water. A sample of trout chow is routinely analyzed for total metals, organochlorine pesticides, and PCBs per OSU AquaTox Standard Operation Procedures (SOPs).

3.0 EXPOSURE SYSTEM

3.1 Route of Administration

Method: Each exposure concentration will be prepared as follows: 1) measure 14 L of sterilized moderately hard reconstituted laboratory water into a cubitainer, 2) measure an additional 900 mL into a large beaker, 3) measure an additional 85 mL into a small beaker, 4) snap ampoule (containing 15 mL LA stock) into large beaker, 5) use some of the water from the small beaker to rinse the ampoule into large beaker, 6) pour contents of large beaker into cubitainer, 7) use remaining water from small beaker to rinse the large beaker out into the cubitainer, 8) cap cubitainer and shake vigorously.

Equipment: Test chambers will be 2.5-gallon glass aquaria. Water within the chambers will be continuously circulated with a microbubble diffuser to provide sufficient mixing and aeration. All construction and materials in contact with test water will be glass, stainless steel, or plastic.

Frequency: Each test chamber will receive 4 L of its respective exposure concentration. This is a static test and no water renewal will occur.

3.2 Dilution Water

Dilution water will be moderately hard reconstituted laboratory water made from deionized water amended with the reagent grade salts ($CaSO_4 \cdot 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$) to achieve a target hardness and alkalinity of 100 mg/L as $CaCO_3$ and 70 mg/L as $CaCO_3$, respectively. The laboratory water will then be pre-sterilized by ozonation (Nutech 03 Ozone Generator Model SC-10; McLean, VA) and ultraviolet (UV) light treatment (Sanitron UV Water Purifier Model A600; Hauppauge, NY). Following sterilization, dissolved organic carbon (DOC) will be added by addition of Suwannee River Natural Organic Matter (NOM; obtained from International Humic Substances Society). NOM will be added, based on a composition of 52.47% carbon, to achieve a nominal concentration of 1 mg/L DOC. A single batch of 200 L of sterilized water will be prepared for use as the control/dilution water in the pilot study.

3.3 Test Temperature

Test temperature will be 12 ± 2°C. Testing will be conducted in a temperature-controlled environmental chamber.

3.4 Test Containers

Test containers will be 2.5 gallon rectangular glass aquaria. Test chambers will contain 4-L of test media. Each test chamber will be fitted with a mixing apparatus, to ensure continuous mixing of the test solution. The mixing apparatus will consist of one horizontal microbubble diffusers on each side of the tank (for a total of two diffusers). The diffusers will ensure consistent mixing and aeration.

3.5 Photoperiod

The photoperiod will be 16-hours light and 8-hours dark, provided by cool-white or daylight illumination.

3.6 Dissolved Oxygen Concentrations

Dissolved oxygen concentrations will be maintained at ≥ 60 percent of saturation. The microbubblers will ensure appropriate dissolved oxygen levels for the entirety of the test.

4.0 TEST DESIGN

4.1 Test Concentrations/Dosages

The test concentrations will be 10, 1, 0.1, and 0.01 billion LA fibers per liter (BFL), plus a dilution water control.

4.2 Number of Test Organisms

A total of 45 organisms will be tested for each concentration and control. Each concentration and control will have 3 replicates (15 fish per replicate).

4.3 Bias Control

To control bias, test chambers will be numbered according to a 5 X 3 randomization sheet and placed in the environmental chamber. Test chambers will also be labeled with the test concentration and replicate number.

4.4 Test Initiation

Fish will be impartially distributed between all of the test chambers until the total number of fish has been achieved in each test chamber.

4.5 Chemical and Physical Monitoring

At a minimum, the following measurements will be made according to the methods laid out in OSU AquaTox SOPs:

1. Hardness, alkalinity, dissolved oxygen, temperature, conductivity, total ammonia, total residual chlorine, pH, calcium, magnesium, sodium, potassium, chloride, sulfate, and dissolved organic carbon will be measured in the control/dilution water at test initiation.
2. Dissolved oxygen, temperature, conductivity, and pH will be measured daily in each exposure concentration.
3. Water samples for LA analysis will be taken (see Section 4.7) at the same time each day from each replicate of each exposure concentration (as explained in Section 4.7). All sampling details (i.e., date, time, investigator) will be recorded in the data packet.

Libby Amphibole (LA) asbestos concentrations will be analyzed at EMSL Analytical (Libby, MT) following sample analysis procedures described under separate cover.

4.6 Biological Monitoring

Any mortality will be recorded once daily and dead fish will be removed immediately. Behavioral effects (i.e., swimming and feeding behavior of test organisms) will be noted daily on the data package, but fish will not be removed until they are considered dead. All biological monitoring will be included in the complete data package together with mortality and other recorded study data (date, time, investigator).

4.7 Analytical Sampling

4.7.1 Filtration of Water Samples

Samples for total and free fiber LA analysis will be collected at test initiation and then at 24 hr periods thereafter. At initiation, samples will be taken directly from the aquaria following the filling of test solution, but prior to the addition of the fish. Samples taken each 24 hours thereafter will be withdrawn directly from the test chamber. Two LA samples will be collected and submitted from each aquaria each sampling event. One method blank (deionized water) and one duplicate sample (selected randomly from 4 LA exposure concentrations) will be taken at each sampling event. Syringe sampling will be the method used at each sampling event

Instructions for the preparation of filters and filtration of the water samples are detailed in OU3 SOP 3A (Rev. 1) titled, Water Sampling with Syringe Filters (Attachment 2). OSU laboratory personnel will be properly trained to perform sampling and filtration procedures by EMSL staff.

Specific volumes to be sampled according to exposure concentration are shown below:

Nominal Exposure Concentration (BFL)	Sample Volume (mL)	Added to	Volume of Moderately Hard RW (mL)		Filter Volume (mL)
10	10	→	990	→	10
1	10	→	90	→	10
0.1	50	→	0	→	10
0.01	50	→	0	→	50
0 (control)	10	→	0	→	10

Each filter holder will be labeled with a pre-numbered sample identification tag which will coincide with the Field Sampling Data Sheet (Attachment B of Attachment 2).

Filter holders will be placed in the filter holder tray (filter side up), placed in a cooler on ice, and shipped to Hygeia Laboratories (address below) within 24 hours of collection.

Hygeia Laboratories
Attn: Kyeong Corbin
82 West Sierra Madre Blvd
Sierra Madre, CA 91024

4.8 Test Duration

The test duration will be 5 days. The test duration of 5 days was chosen to represent an exposure length “greater than the maximum” acceptable period to maintain appropriate water quality for the test organisms in a static system. This time period will also encompass a length of time “greater than the maximum” period where free fibers may begin to clump or bind.

4.9 Test Termination

Immediately following the last analytical sample and last biological reading, fish transfer techniques and equipment will be used to simulate the change of exposure chambers during definitive testing. The preliminary design for transferring the fish from one aquarium to a new aquarium during renewal period begins with siphoning water and waste out of the aquaria (down to a water level which minimizes any stress to the fish). A snugly fit rectangular plastic box (fish corral) will fit snugly down into one side of the aquaria. One side of the fish corral is open where the fish will be corralled into the box from the opposite side of the tank. Once in the box, the box will be lifted out so the fish always remain in water. The outside of the corral will be rinsed with deionized water before moving to the new chamber. The fish corral will then be slightly emerged into the new exposure chamber and fish will be allowed to swim out into the aquaria.

4.10 Quality Criterion

Due to the exploratory nature of the pilot study, no biologically-based quality criterion applies to the conduct of this test.

5.0 DATA ANALYSIS

No specific data analysis relating to biological observations is intended for this study, but a summary of all biological and chemical monitoring conducted during the course of the study will be included in the final report. All analyses will pertain to measurement of free-fiber and total asbestos fiber concentrations and the effect of experimental manipulations on the observed values.

6.0 TEST REPORT

The report will describe the procedures and results of the test and will be signed by the Principal Investigator/Study Director, Quality Assurance Unit, and Study Sponsor. The report will include, but not be limited to, the following:

- Name and address of the test facility;
- Dates of test initiation, completion, and/or termination;
- Objectives of the study as stated in the test protocol, including any changes from the protocol;
- Statistical methods used in data analysis;
- Identification of the test, control, and reference substances (by name, CAS number, or code number) and description of substance purity, strength, composition, stability, solubility, and/or other appropriate characteristics documented by the Study Sponsor (location of documentation shall be specified);
- A description of the methods used during testing;
- A description of the test system used including, where applicable, number of animals used, sex, body weight range, source of supply, species, strain, substrain, age, and procedure for identification;
- A description of the dosage, dosage regimen, route of administration, and duration;
- All analytical results concerning test treatments and water quality;
- A description of all circumstances that may have affected the quality or integrity of the data;
- The name of the Study Director and the names of other scientists, professionals, or supervisory personnel (e.g., task manager, senior biomonitoring technician) involved in the study;
- A summary of all chemical and biological monitoring data collected during the course of the study;
- Signature and date of the Study Director and/or other professionals involved in the study as required by the testing facility or Sponsor;
- The location (s) where all specimens, raw data, and final report are to be stored;

- A statement of Quality Assurance

7.0 RECORD RETENTION

All records will be maintained and archived at OSU AquaTox in accordance with appropriate SOP.

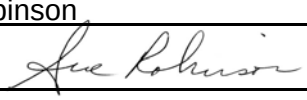
8.0 PROTOCOL AMENDMENTS AND DEVIATIONS

All changes (i.e., amendments, deviations, and final report revisions) of the approved protocol, plus the reasons for the changes, must be documented in writing. The changes will be signed and dated by the Study Director and maintained with the protocol. All protocol amendments must be authorized in advance by the Sponsor.

9.0 PROTOCOL APPROVAL

Sponsor:

Print Name: Sue Robinson

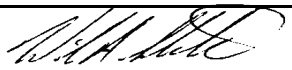
Approval Signature: 

Title: Project Manager

Date: 28 April 2011

Principal Investigator:

Print Name: Bill Stubblefield

Approval Signature: 

Title: Principal Investigator

Date: 28 April 2011

USEPA:

Print Name: Christina Progess

Approval Signature: _____

Title: OU3 Remedial Project Manager

Date: _____

ATTACHMENT A

CHATFIELD METHOD FOR PC FILTER PREPARATION

CHATFIELD TECHNICAL CONSULTING LIMITED

2071 Dickson Road
Mississauga, Ontario
CANADA L5B 1Y8
Telephone: (905) 896-7611
Fax: (905) 896-1930

Presentation
Inter/Micro-94

An Improved Method for Preparation of TEM Specimen Grids from Polycarbonate Membrane Filters Used for Particulate and Fiber Analysis

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Direct-transfer preparation of TEM grids from polycarbonate (PC) filters is a simple procedure in which a carbon extraction replica is produced from the filter surface. Particles are deposited on the surface of the filter by filtration. A portion of the filter is then placed in a carbon evaporator, and a thin film of carbon is deposited on the filter surface. A small area of the carbon-coated filter is placed on a TEM support grid, and the filter medium is dissolved away leaving the original deposit trapped in the carbon film on the TEM grid.

Excessive heating of the surface of PC filters during carbon deposition has been found to render the surface layers of the polymer insoluble in the commonly-used solvents, such as chloroform. Over the last 10 years, PC filters have also varied in their resistance to complete dissolution. It has become clear that the majority of TEM specimen preparations produced by chloroform dissolution of PC filters have a residual layer of undissolved polymer, which reduces particle image contrast and limits the visibility of electron diffraction patterns. Both effects increase the strain on the TEM operator and

introduce the potential for a negative bias in the analysis.

The top micrograph shows an example of a TEM specimen recently prepared by a laboratory accredited by NVLAP for analysis of asbestos in air samples by TEM. Using specimen grids of this quality, the laboratory proceeded with the analysis and reported results. A chrysotile fiber can be found in the area shown in this micrograph, but the thickness of this specimen imposes an unacceptable limitation on particle visibility. This illustrates an extreme example of incomplete filter polymer dissolution, but almost all preparations using chlorinated hydrocarbon solvents show some degree of residual filter polymer, the amount depending on the carbon coating technique and the procedures used for filter dissolution. Assuming that no excessive heating of the filter surface occurred during carbon evaporation, use of a Jaffe washer for 2 hours, followed by a 20 minute treatment in a condensation washer has usually been found to produce TEM grids of acceptable quality. After this treatment, there will be very little further removal of filter polymer even if the condensation washing is extended significantly. The TEM specimens, however, almost always exhibit the "connected pore" phenomenon, which is caused by undissolved polymer.

A new dissolution procedure has been developed which is based on the use of a mixture of 20% 1-2-diaminoethane and 80% 1-methyl-2-pyrrolidone in a Jaffe washer of the conventional wire mesh bridge design. This solvent mixture completely removes the PC filter polymer in a period of 10 minutes. It yields ideal specimen grids, even from filters that have been excessively heated during carbon evaporation. This TEM specimen preparation procedure allows the use of much thinner carbon films, than is the case for the conventional chloroform dissolution procedure, because the distortion of the PC filter observed during dissolution using chloroform does not occur. The new procedure has been incorporated in the International Organization for Standardization Method ISO 10312 (Ambient air - Determination of asbestos fibres - Direct-transfer transmission electron microscopy procedure (Draft) 1993) and in a

new draft method for determination of asbestos fibers in parenteral medicines (Proposed Analytical Method (Draft) Der Verband der Chemischen Industrie, Parenteral Medicines - Determination of asbestos fibres - Direct-transfer transmission electron microscopy procedure, 1994). The bottom micrograph shows an example of a TEM specimen prepared from a polycarbonate filter by the new procedure.

The new dissolution procedure is as follows:

1. prepare a Jaffe washer consisting of a stainless steel mesh bridge in a glass petri-dish;

2. add to the Jaffe washer a sufficient volume of a pre-prepared 20% 1-2-diaminoethane and 80% 1-methyl-2-pyrrolidone mixture, such that the meniscus touches the horizontal underside of the stainless steel bridge;

3. place each TEM grid with a portion of carbon-coated PC filter, carbon side facing upwards, on to the Jaffe washer mesh. Cover the dish and allow to stand for approximately 10 minutes;

4. transfer the stainless steel mesh with the grids to a second, empty petri-dish;

5. to the second petri-dish, add either distilled water or reagent alcohol (ethanol) until the meniscus touches the horizontal underside of the stainless steel bridge. Allow to stand for approximately 10 minutes;

6. remove the stainless steel bridge from the Jaffe washer, place it on a paper towel, and allow to dry. The drying process can be accelerated by absorbing the excess water or ethanol from the underside of the mesh using paper towel.

The choice of distilled water or reagent alcohol as the final washing

solvent depends on the application or the preference of the analyst. If it is required to retain water-soluble particle species such as gypsum on the final TEM specimens, then alcohol must be used. For water sample analysis, or for indirect TEM specimen preparations from air samples in which water has been the dispersal medium, either water or reagent alcohol may be used. For direct-transfer air sample preparations in which it is desired to remove gypsum fibers from the preparation, water should be used. In analyses of air samples for asbestos, thin gypsum fibers may be present in large numbers, and these can slow down the TEM examination because each fiber must be identified. Selective removal of gypsum from such samples is advantageous, in that the TEM examinations proceed more rapidly after non-asbestos fibers have been removed, even if carbon replicas of the original particles still remain visible in the image.

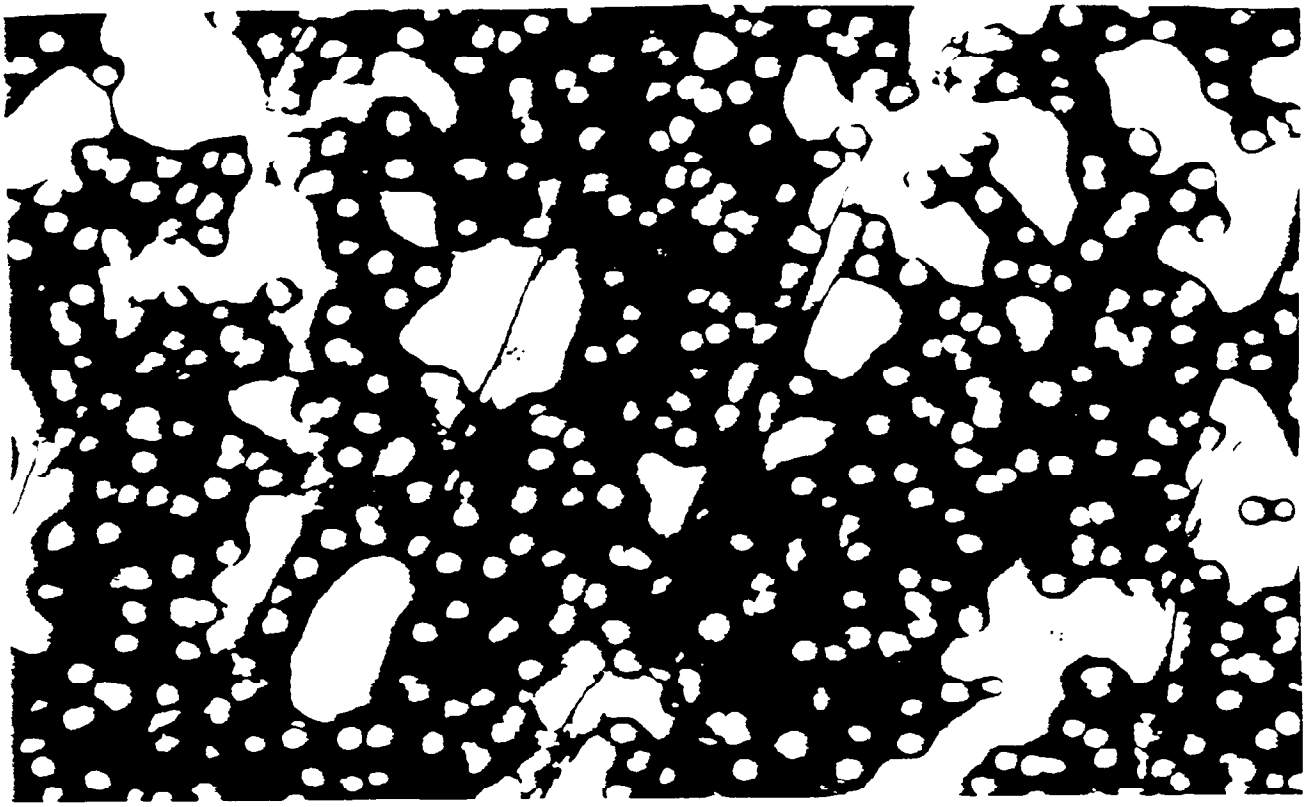
Some types of copper grid are slightly attacked by the solvent. It is not recommended, nor is it necessary, to allow grids to remain in contact with this solvent for more than approximately 10 minutes, and substantially less dissolution time may be sufficient in some cases. A slight blue color in the solvent after use will indicate if any chemical attack on the grids has occurred, but this has not presented any problems. If this attack is of concern, or if for operational reasons the grids must remain in contact with the solvent for periods of time longer than approximately 15 minutes, gold grids may be used instead of copper and are not attacked by the solvent.

Using the new procedure, dissolution occurs so rapidly that interference colors, created by the rapidly thinning polymer layer, can be observed only a few seconds after dissolution commences. No distortion of the shape of the carbon-coated PC filter portion is seen during dissolution. Starting from a PC filter, ideal TEM specimens can be available for TEM analysis in a period of less than 30 minutes. This is a significant improvement over the conventional methods for preparation of TEM specimens from PC filters. It also represents considerable savings of time over the MCE preparation methods,

bearing in mind the additional time requirements for collapsing of the MCE filter and plasma etching.

Figures: Top: TEM micrograph showing the appearance of a specimen prepared from a 0.2 micrometer pore size PC filter on which chrysotile fibers had been collected. This shows the effects of overheating during carbon evaporation, and incomplete dissolution of the filter polymer by chloroform. Magnification 11,000

Bottom: TEM micrograph showing the appearance of a similar specimen prepared using the new dissolution procedure. Magnification 11,000



ATTACHMENT B

ANALYSIS OF WATER SAMPLES FOR ASBESTOS BY TEM

FOR USE AT LIBBY OPERABLE UNIT 3 ONLY

LIBBY OU3 MODIFICATION 1 TO ISO 10312 METHOD ANALYSIS OF WATER SAMPLES FOR ASBESTOS BY TEM

Revision 0

Date: May 21, 2009

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Remedial Project Manager	Bonita Lavelle, USEPA RPM	5/22/09
Modification Author	William Brattin, SRC	5/22/09

Revision	Date	Reason for Revision
0	May 21, 2009	--

1.0 PURPOSE

The purpose of this document is to provide modifications to ISO Method 10312 for use at the Libby Superfund Site Operable Unit 3 in the analysis of water samples for Libby Amphibole (LA) by transmission electron microscopy (TEM).

2.0 RESPONSIBILITIES

The Laboratory Director is responsible for ensuring that water samples provided to the laboratory for analysis are prepared and analyzed in accord with the requirements of this modification. It is also the responsibility of the Laboratory Director to communicate the need for any deviations from the modification to the appropriate U.S. Environmental Protection Agency (USEPA) Region 8 Remedial Project Manager or Regional Chemist.

3.0 EQUIPMENT

Sample Preparation

- Sonication device
- Oxygen tank
- Ozone generator
- Plastic and glass tubing

Sample Filtration

- NVLAP-compliant High Efficiency Particulate Air (HEPA) hood
- Particle-free water
- Forceps
- Disposable 47 mm filter funnels
- Side arm filter flask
- Mixed Cellulose Ester (MCE) filters, 47 mm diameter, 0.2 μm and 5.0 μm pore size
- Storage container for filters

Grid preparation and Analysis by TEM

All equipment needed for TEM grid preparation and analysis by TEM analysis is detailed in ISO 10312.

4.0 MODIFICATION SUMMARY

Samples of water from field sampling or laboratory-based studies are transmitted to a qualified laboratory for analysis of asbestos. At the laboratory, aliquots of water are filtered, and the filters are analyzed by TEM in accord with ISO 10312 as specified in the applicable Sampling and Analysis Plan. All results are expressed in units of million fibers per liter (MFL).

5.0 SAMPLE PREPARATION

The project-specific Sampling and Analysis Plans should specify if and how water samples should be prepared for analysis. In some cases, no preparation is needed other than ensuring the sample is well-mixed before filtration. In other cases, it may be appropriate to use sonication to disperse clumps of fibers that may be present, or to use sonication and ozone treatment combined, as detailed in EPA Method 100.1 Step 6.2, especially in samples where microbial growth or other organic matter may be present.

6.0 FILTER PREPARATION

After sample preparation (if needed), one or more aliquots of water from each sample will be filtered through 47 mm MCE filters with 0.2 μm pores, using a backing filter with pore size of 5 μm. The volume of water filtered should be selected to provide a filter loading of about 100-1000 asbestos structures per mm^2 on the filter.

For water samples in which it is possible to estimate the concentration before analysis (e.g., samples from a laboratory-based toxicity test), the appropriate volume may be estimated as follows:

$$\text{Volume (mL)} = \frac{\text{Target Loading (s/mm}^2\text{)} \cdot \text{Effective Filter Area (mm}^2\text{)}}{\text{Expected Concentration (s/mL)}}$$

For example, assuming an effective filter area of 1295 mm^2, for the analysis of a sample with an expected concentration of 100 MFL (1E+05 s/mL), a loading of about 500 s/mm^2 would be expected after filtration of about 6 mL.

For water samples for which the concentration can not be reasonably estimated before analysis (e.g., most field samples), then it may be necessary to prepare a series of filters, each with a different volume of water. Typically, this will be done by filtering aliquots of 100 mL, 30 mL, and 10 mL of the sample. Select the filter from the dilution series yielding the largest possible application volume which does not result in an overloaded sample (> 2000 structures per mm^2). If the 10 mL aliquot is overloaded, the laboratory shall prepare a dilution of the sample by removing 5 mL of the remaining volume and diluting to 100 mL. From this secondary dilution, prepare a second series of filters using 60 mL, 20 mL, and 6 mL (corresponding to 3.0 mL, 1.0 ml, and 0.3 mL of the original suspension).

7.0 TEM ANALYSIS

Remove a wedge of about ¼ of the sample filter. Prepare at least 4 grids for TEM analysis as detailed in ISO TEM method 10312, also known as ISO 10312:1995(E). Utilize a minimum of 2 grids (typically 3) for analysis, distributing grid openings examined distributed approximately evenly across the grids. Archive the remaining grid(s) and the remaining filter.

Counting rules

All water samples submitted for asbestos analysis by TEM will be analyzed in basic accord with the ISO 10312 counting protocols, with all applicable Libby site-specific Laboratory Modifications, including the most recent versions of modifications LB-000016, LB-000019, LB-000028, LB-000029, LB-000030, LB-000066, and LB-000085.

Stopping Rules

The target analytical sensitivity for sample analysis should be specified in the project-specific SAP. In the absence of such specification, the default target analytical sensitivity for asbestos in water is 50,000 f/L (0.05 MFL). Stopping rules for these analyses are as follows:

1. Calculate the number of grid openings (GOs) needed to achieve the target sensitivity.
2. Count a minimum of 2 GOs in each of 2 grids.
3. Continue counting until one of the following stopping rules is achieved:
    a. The target sensitivity is achieved
    b. A total of 50 Libby amphibole (LA) structures have been counted
    c. A total area of 0.5 mm^2 (usually about 50 GOs) has been examined
4. When one of these rules has been achieved, finish counting the final GO, then stop.

Data Recording and Electronic Data Deliverable

Standard Analysis

Unless otherwise specified in the project-specific SAP, all amphibole structures (including not only LA but all other amphibole asbestos types as well) that have appropriate Selective Area Electron Diffraction (SAED) patterns and Energy Dispersive X-Ray Analysis (EDXA) spectra, and having length ≥ 0.5 μm and an aspect ratio (length:width) ≥ 3:1, will be recorded on the most recent version of the Libby site-specific laboratory bench sheets and EDD spreadsheet ("TEM Water EDD.xls"). Data recording for chrysotile, if observed, is not required.

Rapid Turn-Around Analysis

In some cases, the project-specific SAP may specify that some water samples shall be analyzed using a “rapid turn-around” protocol. The rapid turn-around protocol differs from the standard analysis as follows:

1. Quantitative measurement of length and width is not required for structures that can be readily classified as countable by eye. Measurements may be necessary for structures that are near the size cutoffs for counting (i.e., length close to 0.5 μm, aspect ratio close to 3:1).
2. Recording of individual structure dimensions and characteristics is not required.
3. Electronic documentation of EDS spectra or SAED patterns is not required.
4. Classification of structures in accord with Libby Laboratory Modification #LB-00066 is not required.

The total number of LA structures observed in each grid opening should be recorded on the most recent version of the Libby site-specific laboratory bench sheets and EDD spreadsheets ("Rapid TEM Water EDD.xls").

8.0 QUALITY CONTROL

The project-specific Sampling and Analysis Plan should specify the types and number of laboratory quality control (QC) samples that should be prepared during the project. In the absence of information in the sampling and Analysis Plan, default guidelines for QC samples are provided in Table 1. This table includes default requirements on the frequency that these QC analyses should be performed, how samples will be selected for QC analyses, the acceptance criteria and corrective actions for these analyses. It is the responsibility of the laboratory manager to ensure that QC requirements are met.

9.0 REFERENCES

International Organization for Standardization (ISO). 1995. Ambient Air – Determination of asbestos fibers – Direct-transfer transmission electron microscopy method. ISO 10312:1995(E).

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TABLE 1
LABORATORY QUALITY CONTROL SAMPLE DEFAULT REQUIREMENTS [a]

Lab QC Type & Description	Analysis Frequency [b]	Acceptance Criteria	Corrective Action(s)
Lab Blank A filter that is prepared using laboratory water.	1% (1 per 100 analyses)	No asbestos structures observed in an analysis of 10 GOs.	The laboratory shall immediately investigate the source of the contamination and take steps to eliminate the source of contamination before analysis of any investigative samples may continue.
Repreparation Prepared by applying a second aliquot of sample water to a new filter, which is then prepared and analyzed in the same fashion as the original filter.	2% (1 per 50 analyses) See note [c]	No more than 5% of the original-repreparation pairs are statistically different from each other at the 90% confidence interval. See note [d]	A senior laboratory analyst shall determine the basis of the discordant results, and take appropriate corrective action (e.g., re-training in sample and filter preparation, counting rules, etc).
Recounts **Recount Same.** A re-examination the same grid openings as were evaluated in the original analysis by the same microscopist who performed the initial examination. **Recount Different.** A re-examination the same grid openings as were evaluated in the original analysis by a different microscopist within the same laboratory who performed the initial examination.	2% (1 per 50 analyses) See note [c]	See Libby Laboratory Modification LB-000029	A senior laboratory analyst shall determine the basis of the discordant results, and take appropriate corrective action (e.g., re-training in counting rules, etc).
Interlabs A re-examination the same grid openings as were evaluated in the original analysis by a different laboratory who performed the initial examination.	1% (1 per 100 analyses) See note [e]	See Libby Laboratory Modification LB-000029	A senior laboratory analyst at the interlaboratory will contact the originating laboratory to discuss the basis of the discordance. As appropriate, each laboratory will take appropriate corrective action (e.g., re-training in counting rules, etc).

[a] Unless specified otherwise in the project-specific sampling and analysis plan or quality assurance project plan.
[b] When calculating the number of QC analyses required for a project, round up to the nearest whole number.
[c] To be selected by the laboratory in accord with the procedures in Attachment 1 in Libby Laboratory Modification LB-000029.
[d] See Attachment 4 in Libby Laboratory Modification LB-000029 for details on performing this statistical comparison.
[e] To be selected by EPA (or EPA's technical contractor) in accord with the procedures in Attachment 2 in Libby Laboratory Modification LB-000029.

ATTACHMENT 2

WATER SAMPLING WITH SYRINGE FILTERS

OU3 SOP 3A (Rev. 1)

Date: April 27, 2011

OU3 SOP 3A (Rev. 1)

Title: WATER SAMPLING WITH SYRINGE FILTERS

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Remedial Project Manager		4/27/11
SOP Author	WJ Britton	4/27/11

REVISION HISTORY

Revision Number	Date	Significant Revisions
0	03/18/2011	--
1	4/27/2011	Add settling step to remove coarse particulates Add detail on tightening of filter holders Changed to 0.2 um pore size filters to decrease time required for filtration

1.0 INTRODUCTION

This Standard Operating procedure (SOP) describes a method for collection and filtration of water samples for the analysis of free (un-bound) Libby Amphibole (LA) asbestos. This SOP is applicable to site water or laboratory water samples collected as part of the Remedial Investigation in Operable Unit 3 (OU3) of the Libby Asbestos Superfund Site.

2.0 HEALTH AND SAFETY WARNING

All personnel engaged in sampling or testing of water containing LA must follow health and safety protocols described in the appropriate health and safety plan. Inhalation exposure to asbestos during field or laboratory work may increase the risk of lung cancer, mesothelioma, asbestosis, and other respiratory diseases.

3.0 RESPONSIBILITIES

This section presents a brief definition of field roles, and the responsibilities generally associated with them. This list is not intended to be comprehensive and often, additional personnel may be involved. Project team member information will be included in project-specific plans (e.g., work plan, field sampling plan, quality assurance plan, etc.), and field personnel will always consult the appropriate documents to determine project-specific roles and responsibilities. In addition, one person may serve in more than one role on any given project.

Project Manager: Specifies the site-specific field or laboratory sampling program, with input from other key project staff and applicable oversight agencies.

Quality Control Manager: Overall management and responsibility for quality assurance and quality control (QA/QC). Specifies QA/QC procedures for the sampling and analytical methods, performs project audits, and ensures that data quality objectives are fulfilled.

Field Team Leader (FTL) and/or Geologist, Hydrogeologist, or Engineer: Implements the sampling program, supervises other sampling personnel, and ensures compliance with SOPs and QA/QC requirements. Prepares daily logs of field or laboratory activities.

Sampling Technician (or other designated personnel): Assists the FTL in the collection and handling of samples. Performs the actual sample collection, packaging, and documentation (e.g., sample label and log sheet, chain-of-custody record, etc).

4.0 SYRINGE FILTRATION PROCEDURE

4.1 Background

Measurement of LA in site or laboratory waters is complicated by the finding that if the water is not completely sterile, organic matter associated with microbial contamination tends to form, which tends to cause the fibers to clump together (EPA 1983a). This causes two effects: a) asbestos fibers that are clumped together are difficult to observe and count using transmission electron microscopy (TEM), and this may lead to a decrease in estimated concentration of LA in the water, and b) fibers within clumps of organic matter tend to adhere to the walls of the sample bottles, thus decreasing the concentration of fibers in the water. The magnitude of these effects is time-variable, and depends on the amount of organic matter present and the time the sample is held before filtering. Both phenomena (fiber clumping, fiber adherence to container walls) have been observed in studies performed to date by EPA at the Libby OU3 site, including a juvenile rainbow trout toxicity test performed using site waters in 2009, and analysis of surface water samples collected at stream sampling station LRC-06 in July 2009.

EPA developed Analytical Method 100.1 (EPA 1983b) for the analysis of <u>total</u> asbestos in water. This method involves treating the water sample with ozone, ultraviolet light, and sonication before filtration. This treatment oxidizes organic material that is present in the water or on the walls of the sample bottle, destroying the material that causes clumping and binding of fibers. Based on studies performed by EPA, this treatment allows good recovery of fibers under a variety of starting conditions.

In some cases, it may also be necessary to measure the concentration of <u>free</u> asbestos in water samples in order to achieve data quality objectives, where “free asbestos” refers to asbestos fibers that exist in the water but are not associated with clumps of organic material.

When seeking to measure the concentration of free asbestos in water, the details of how the water is handled before filtration may be important. For example, if the water sample is placed in a bottle for transport to the laboratory for filtration, it is possible that additional clumping might occur and/or that binding of clumps to the bottle wall might occur before filtration occurs. If so, this could yield results that are not representative of the true concentration of free asbestos in the water at the time of sampling.

One way to avoid this potential problem is to filter the water sample directly at the site of collection, avoiding the lag time needed to transport sample bottles to the laboratory. The purpose of this SOP is to describe a method for the preparation of water filters at the site of collection (field, laboratory) using a syringe filter technique.

4.2 Syringe Filter Sample Collection

4.2.1 Sampling Equipment

The key equipment needed to collect water syringe filter samples is:

- One liter HDPE bottles for collecting water samples in the field
- Plastic syringes, with Luer-Lok fitting, adequate to hold the volume of water specified in the project-specific SAP (typically 10-50 mL)
- Filter holders for 25 mm diameter filter (e.g., Millipore Swinnex SX0002500, or equivalent)
- 25-mm diameter polycarbonate filters with 0.2-um pore size
- 25-mm diameter mixed cellulose ester filters with 5-um pore size
- 25-mm cellulose backing pads
- Tweezers for handling filters
- Filter holder trays for sample storage
- Pre-numbered self-adhesive sample identification labels
- Vinyl electrical tape
- FSDS (see Attachments A and B)

In addition, the following equipment is needed to support the documentation of sampling locations and for sample handling after collection:

- Field notebook, indelible marker
- Global Positioning System (GPS) unit
- Digital Camera
- Chain of custody sheets
- Coolers

4.2.2 Preparing the Filter Holders

Prior to sample collection and filtration, prepare an adequate number of filter holders for use as follows:

1. Unscrew the filter holder
2. Using tweezers, carefully place a 25-mm cellulose backing pad on the filter support in the lower half of the filter holder. On top of this, place a 25-mm MCE filter with 5 um pore size, and on top of that place a 25-mm polycarbonate filter (0.2-um pore size). Ensure that the shiny side of the PC filter is facing upward.
3. Ensure that the Teflon gasket is properly located in the upper portion of the filter holder.
4. Carefully screw the upper portion and the lower portion of the filter holder together. Stop when the halves are firmly finger tight.

4.2.3 Filtration Protocol

Where multiple sampling stations exist along a moving water source (i.e., a creek or drainage channel), water samples generally will be collected from downstream to upstream locations, to minimize the effect of sampling activities on the samples collected.

For collecting syringe filter samples, the procedure outlined below shall be followed.

1. Don appropriate health and safety equipment (if needed)
2. Immediately before sampling, re-tighten each filter holder by firmly grasping the two halves with both hands and twisting firmly.
3. Seal the two filter holder halves together with electrical tape while maintaining tension on the tape.
4. For field samples from OU3, collect a sample of water from the test location in a one liter HDPE bottle. Allow to settle for 2-3 minutes to clear any coarse particulates that may be present. Then, fill a syringe of the appropriate volume (as specified in the project-specific SAP) with water from the upper portion of the bottle.
   For water samples collected from laboratory-based toxicity tests, fill the syringe directly from the test chamber. No settling step is required.
5. Turn the syringe upward, and tap to cause any air bubbles to rise to the open end
6. Push the syringe plunger upward, expelling all air bubbles, and adjusting the volume in the syringe to the sample volume specified in the project-specific SAP (typically 10-50 mL).
7. Attach a filter holder containing a 25-mm polycarbonate filter with 0.1-um pore size.
8. Turn the syringe with filter holder downward, and hold as nearly vertical as possible.
9. Using firm pressure, press the syringe plunger downward, forcing all of the water in the syringe through the filter. Do not exert excess pressure, since this may cause water to leak past the filter. The time require to filter a sample is expected to be about 1 minute per 10 mL filtered.
10. Detach the filter holder from the syringe. Maintain the filter orientation. Re-attach a clean 25-mL plastic syringe, with the plunger withdrawn to the 20-mL mark. Holding vertically, press the plunger down, forcing 20 mL of air through the filter and filter holder. Detach the syringe, withdraw the plunger to 20 mL, and force an additional 20 mL of air through the filter and filter holder. This will expel any loose water droplets from the filter holder.
11. Attach a pre-numbered sample identification tag to the filter holder and to the FSDS that records all the relevant data for the sample collected.

4.2.4 Sample Handling

Maintain the filter in the syringe filter holder. Seal the inlet and outlet of the filter holder with vinyl electrical tape to prevent evaporation.

Place the filter holder in a filter holder tray, filter-side up. Place the tray in a cooler, maintaining the filter-side up orientation. Transport or ship the filter holders to the designated analytical laboratory within 24 hours of collection.

4.3 Documentation

At each surface water station, sample details will be recorded on a field sample data sheet (FSDS) form. For field samples, the FSDS provided as Attachment A should be used. For water samples collected as part of a laboratory-based toxicity test, the FSDS provided as Attachment B should be used.

In addition, a field logbook will be maintained in accordance with SOP-9. Data items that shall be recorded in the field logbook include:

a. Project identification (e.g., OU3 RI Phase 4B)
b. Location and sample identification, including global positioning system coordinates for field samples (see SOP-11)
c. Date and time of sample collection
d. Any deviations from this SOP, and any field conditions that may influence sample quality or relevance

When the sampling activity is completed, the record will be checked by the Project Manager or his/her designee, and the original record will be placed in the project file.

5.0 QUALITY ASSURANCE AND QUALITY CONTROL

Field blank and field duplicate samples will be collected at the frequencies documented in the project-specific sampling and analysis plan.

Field blanks will be prepared by bringing an adequate volume of filtered and deionized (FDI) laboratory water to the sample collection site, and filtering the specified volume (usually10 mL) through the filter holder.

Field duplicates will be prepared by collecting and filtering a second sample of the same volume from the same location at approximately the same time (within 10 minutes) as the original sample.

6.0 DECONTAMINATION

All syringes and filter holders used in the sampling process shall either be new (un-used) or else shall be thoroughly washed and decontaminated prior to field use and between sample events.

7.0 TRAINING AND PROFICENCY DEMONSTRATION

All sampling technicians must perform a practice round and demonstrate proficiency prior to collection of authentic field or laboratory syringe filter samples. This will be done as follows:

1. Prepare four syringe filter holders as described in Section 4.2.2
2. Prepare a suspension of India ink by adding about 2.5-10 uL (depending on the strength of the ink) to one liter of water.
3. Pass 20 mL of the Ink dilution through each of the four filters, as described in Section 4.2.3.
4. Remove the filters from the filter holders, and allow to air dry
5. Photograph the filters using a digital camera and transmit the image by e-mail to the analytical laboratory in Libby (mobileasbestoslab@emsl.com) for evaluation.

8.0 REFERENCES

EPA. 1983a. Development of Improved Analytical Techniques for Determination of Asbestos in Water samples. Report prepared for the U.S. Environmental Protection Agency, Environmental Research Laboratory, Office of Research and Development, Athens, GA, by the Ontario Research Foundation, Mississauga, Ontario. EPA-600/4-83-042. September, 1983.

EPA. 1983b. Analytical Method for Determination of Asbestos in Water. U.S. Environmental Protection Agency, Environmental Research Laboratory, Office of Research and Development, Athens GA. EPA-600/4-83-043. September, 1983.

ATTACHMENT A

FIELD SAMPLING DATA SHEET FOR SYRINGE FILTER SAMPLES OF SITE WATERS

LIBBY OU3 FIELD SAMPLE DATA SHEET
SURFACE WATER AND SEDIMENT

Station ID: _____ **Sampling Date**: _____
Field Logbook ID: _____ **Logbook Page No**: _____
GPS Coordinate System: UTM Zone 11 North, NAD83 datum, meters
***For New Stations Only*:** **X coord:** _____ **Y coord:** _____ **Elev:** _____
Sampling Team: **MWH** **Samplers Initials:** _

WATER QUALITY PARAMETERS (if applicable)

Time Measured (hh:mm)	Temp. (°C)	pH	Specific Conductan (mS/cm Auto-comp @ 25°C)	Diss. O_2 (mg/L)	ORP (mV)	Turbidity (NTU)

SAMPLE COLLECTION

Index ID	*AFFIX LABEL HERE*	**Sampling Time:_____** **Sample Type: Field Sample** **Media :** ☐ **Sediment** ☐ **SW (aqueous)** ☐ **SW (filter) - Vol. Applied_____mL**	**Sampling Method** (if applicable)**:** **Grab or Composite** **# of Composites:_____** **Sampling Depth: Top (in)_____** **Bot (in)_____**
Index ID	*AFFIX LABEL HERE*	**Sampling Time:_____** **Sample Type: Field Sample** **Media :** ☐ **Sediment** ☐ **SW (aqueous)** ☐ **SW (filter) - Vol. Applied_____mL**	**Sampling Method (if applicable):** **Grab or Composite** **# of Composites:_____** **Sampling Depth: Top (in)_____** **Bot (in)_____**
Index ID	*AFFIX LABEL HERE*	**Sampling Time:_____** **Sample Type: SP FD MS MSD PE FB TB EB** **Media :** ☐ **Sediment** ☐ **SW (aqueous)** ☐ **SW (filter) - Vol. Applied_____mL**	**Sampling Method (if applicable):** **Grab or Composite** **# of Composites:_____** **Sampling Depth: Top (in)_____** **Bot (in)_____**

COMMENTS

Note: **FS** Field Sample **SP** Field Split Sample **FD** Field Duplicate Sample
TB Trip Blank Sample **MS** Matrix Spike Sample **MSD** Matrix Spike Duplicate Sample
FB Field Blank Sample **EB** Equipment Decon Blank Sample **PE** Performance Evaluation Sample

Field Data Entered by:		**Field Entries Checked by:**	

ATTACHMENT B

FIELD SAMPLING DATA SHEET
FOR SYRINGE FILTER SAMPLES FROM
LABORATORY-BASED TOXICITY STUDIES

LIBBY OU3 FIELD SAMPLE DATA SHEET
SYRINGE FILER SAMPLES FROM TOXICITY STUDIES

Testing laboratory:_____________________________
Study name: ____
Samplers Initials: ___________________

SAMPLE DATA

	Sample Number	Sample Type	Sample Information
Index ID	*AFFIX LABEL HERE*	Field sample Field Blank Field duplicate	Test Chamber:________________ Nominal Concentration:_______________ Sample day:_____________ Sample time:___________________ Sample volume (mL):______________
Index ID	*AFFIX LABEL HERE*	Field sample Field Blank Field duplicate	Test Chamber:________________ Nominal Concentration:_______________ Sample day:_____________ Sample time:___________________ Sample volume (mL):______________
Index ID	*AFFIX LABEL HERE*	Field sample Field Blank Field duplicate	Test Chamber:________________ Nominal Concentration:_______________ Sample day:_____________ Sample time:___________________ Sample volume (mL):______________
Index ID	*AFFIX LABEL HERE*	Field sample Field Blank Field duplicate	Test Chamber:________________ Nominal Concentration:_______________ Sample day:_____________ Sample time:___________________ Sample volume (mL):______________
Index ID	*AFFIX LABEL HERE*	Field sample Field Blank Field duplicate	Test Chamber:________________ Nominal Concentration:_______________ Sample day:_____________ Sample time:___________________ Sample volume (mL):______________

COMMENTS

Data Entered by:		**Entries Checked by:**	